

# 3D Printed Hyaluronic Acid Hydrogels using free form fabrication technique for Tissue Engineering



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## Abstract

Three-Dimensional (3D) printing is a manufacturing method in which objects, such as scaffolds, are made by fusing or depositing various materials in layers. The biomedical applications of 3D printing are numerous and continue to grow exponentially. In the present study we designed, developed and fabricated a chemically cross linked honeycomb scaffold using Hyaluronic acid, Gelatin, Alginate, and single walled carbon nanotubes (CNTs) for use in tissue engineering and tissue regenerative applications. All three polymers have been used in various tissue engineering applications from liver regeneration to interocular lens (IOL) replacements. Cross linking of the homogenous polymers were done in a calcium chloride ( $\text{CaCl}_2$ ) bath. Preliminary tests on non-3D printed hydrogel showed increased mechanical stability and a solid structure when compared to the brittle, and mechanically unstable non cross linked hydrogels.

## 1. Method and Materials

### 1.1 Materials

High molecular weight Hyaluronic Acid (HA), Gelatin (Gel), and Alginate Acid (AG) were used to create the hydrogel. Calcium Chloride ( $\text{CaCl}_2$ ) is used as the crosslinking agents. All materials used are biocompatible<sup>1,2</sup>

Concentrations:

- HA = 2% and 3%
- Gel = 2% and 3%
- AG = 2% and 3%
- Single Walled Carbon Nanotubes (CNTs) = 100-200mg
- $\text{CaCl}_2$  = 5% (in bath)

### 1.2 Methods

0.4 g and 0.6g of HA, Gel and AG were, respectively, dissolved in 20mL of deionized water ( $\text{dH}_2\text{O}$ ). All three polymers were vortexed together until a homogenous polymer mixture was made. Polymers were left in a plastic mold overnight before crosslinking, for 30 minutes in a 20ml bath of 5%  $\text{CaCl}_2$

### 1.3 Chemistry

- This work takes advantage of replacement chemistry. Ca is more reactive than the sodium (Na). Resulting in the replacement of Na to Ca within the chains of AG. The Ca then attaches to oxygen of the Gel and HA resulting in crosslinking of the polymer chains via ionic bonding<sup>3</sup>.
- The polymer chains arrange themselves into an “egg box” model.

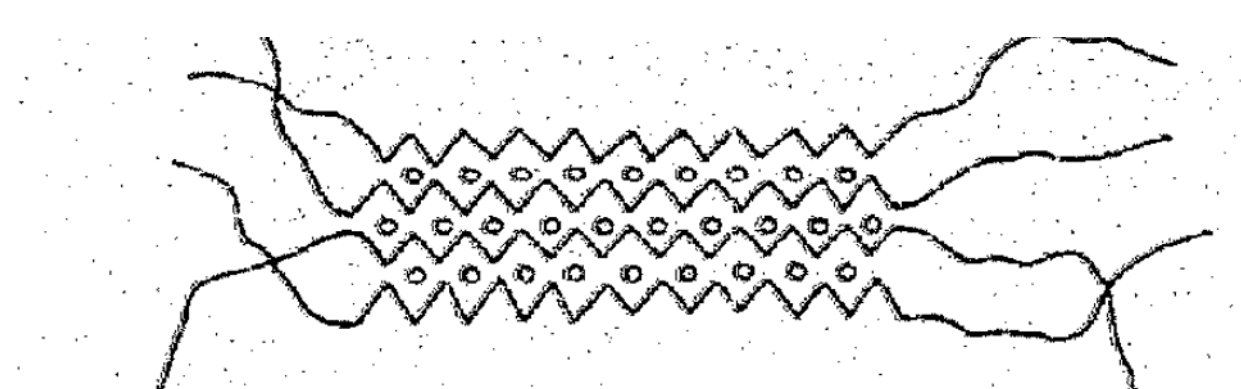


Figure 1: Simple representation of egg box model

## 2. Results

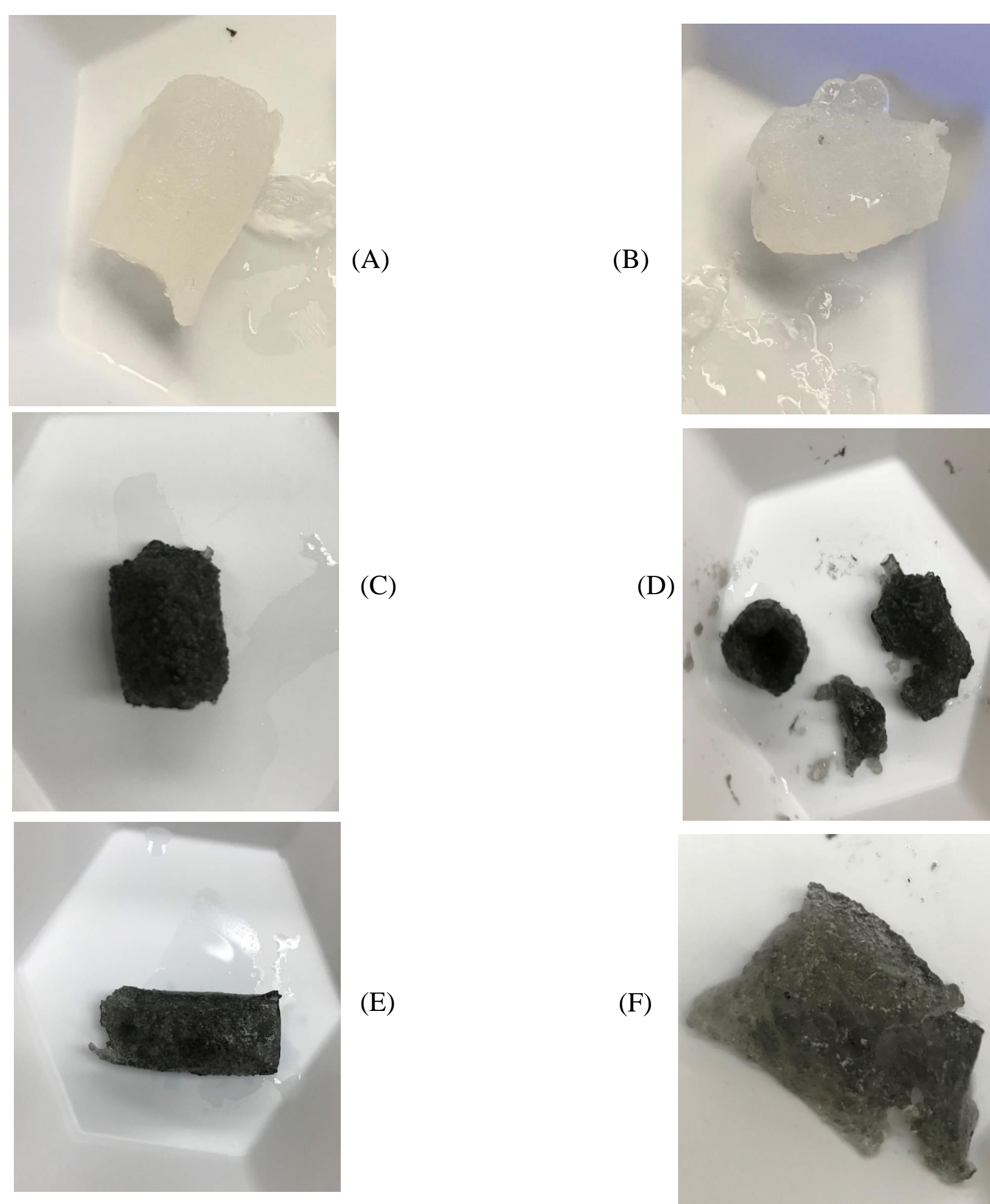


Figure 2: Optical images of : (A) Crosslinked HA, Alginate, Gel hydrogels without SWCNT; (B) Uncrosslinked HA, Alginate, Gel hydrogels without SWCNT; (C) Crosslinked HA, Alginate, Gel hydrogels with 100mg of SWCNT; (D) Uncrosslinked HA, Alginate, Gel hydrogels with 100mg of SWCNT; (E) Crosslinked HA, Alginate, Gel hydrogels with 200mg of SWCNT; (F) Uncrosslinked HA, Alginate, Gel hydrogels with 200mg of SWCNT

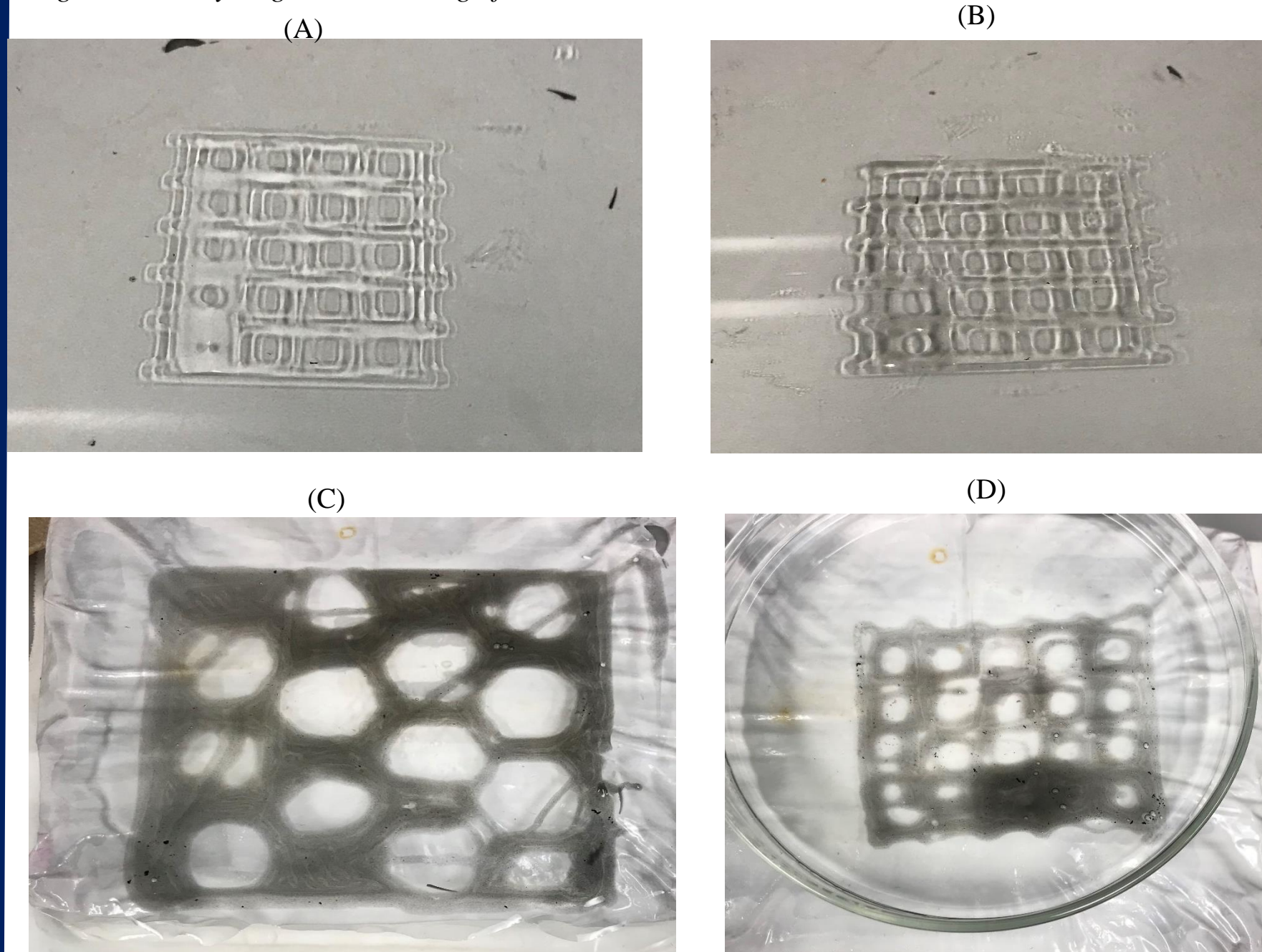


Figure 3: Optical photos of 3D printed scaffold using free form fabrication. (a): 3% solution w/0 SWCNT; (b) 3% solution w/ 100mg of SWCNT; (c) 2% solution w/ 100mg of SWCNT; (d) 2% solution with 200mg of SWCNT.

## 4. 3D printing

Using SolidWorks the scaffolds were modeled to resemble regular 3D lattices (Right) and the honeycomb structure found in corneal endothelium (left)

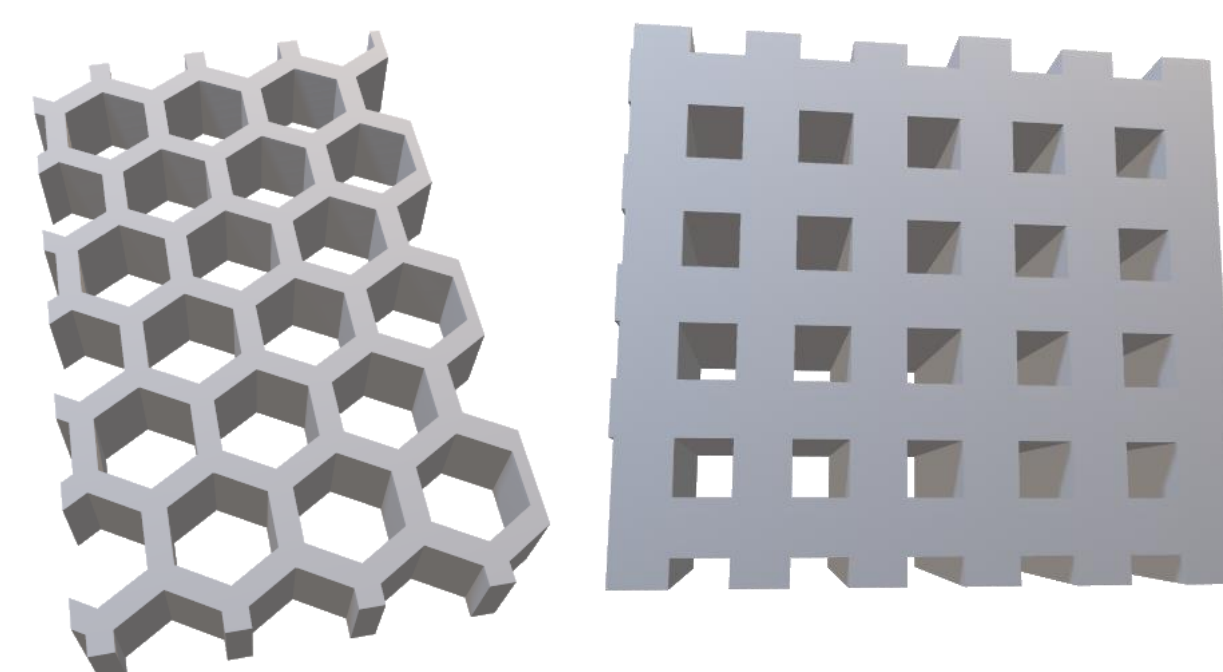


Figure 4: Designed 3D model of honeycomb and lattice structure for 3D printing. Using SolidWorks

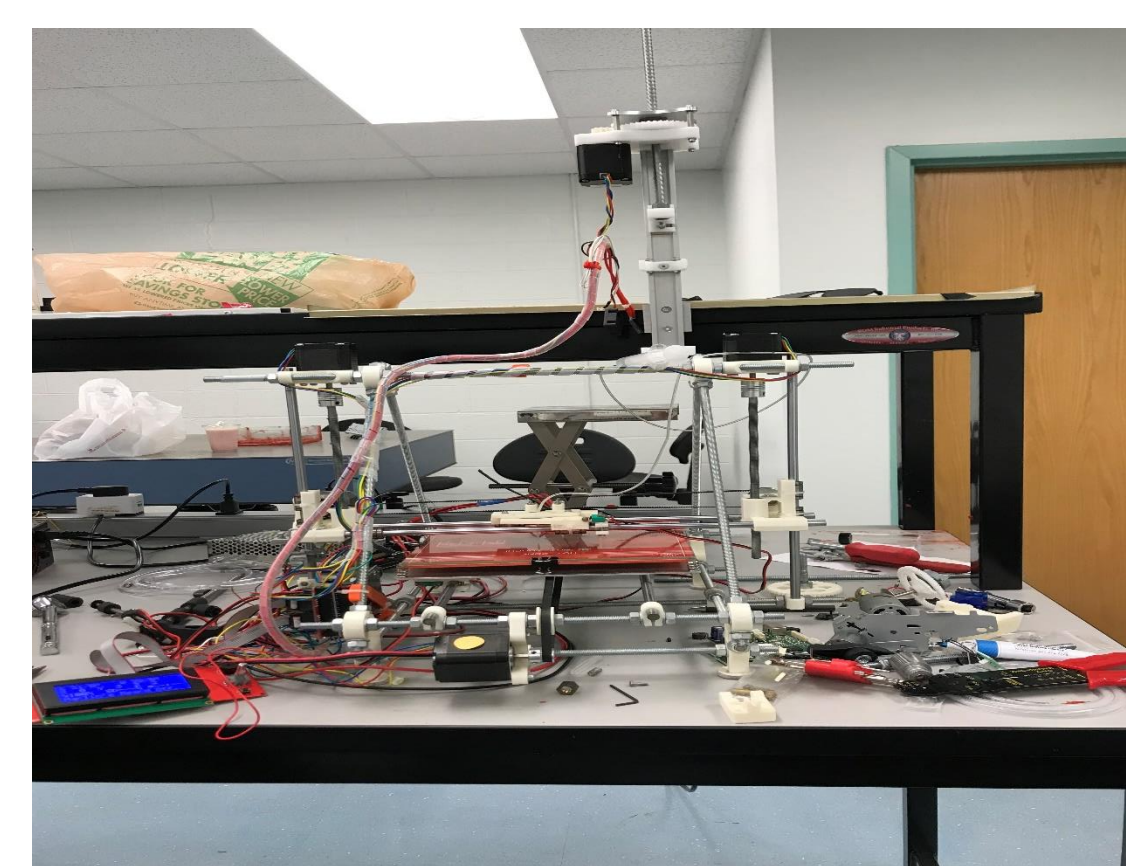


Figure 5: “Frankenstein” custom made 3D printer used for

## 5. Conclusion

We have been successful in developing a free standing hydrogel using only biocompatible materials and simple replacement chemistry. After 3D printing we plan to test CHO cell viable compared to basic cells culture techniques.

## 6. Acknowledgements

- University of Bridgeport

## 7. References

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